ODOR SENSITIVITY AND THE PRESENCE OF P-CRESOL

By

Jessica Mi McKinney

Approved:

Irene N. Ozbek
Professor of Psychology
(Chair)

Amye R. Warren
Professor of Psychology
(Committee Member)

Stefanie R. Whitson
Professor of Chemistry
(Committee Member)

Jeffery Scott Elwell
Dean of the College of Arts and Science

A. Jerald Ainsworth
Dean of the Graduate School

ODOR SENSITIVITY AND THE PRESENCE OF P-CRESOL

By

Jessica Mi McKinney

A Thesis Submitted to the Faculty of the University
of Tennessee at Chattanooga in Partial
Fulfillment of the Requirements
for the Degree of Master of
Science in Psychology

The University of Tennessee at Chattanooga
Chattanooga, TN

May 2013

ABSTRACT

Olfactory function declines throughout the different stages of kidney disease. End-stage renal disease (ESRD) patients have experienced improved olfactory function after dialysis, leading some researchers to suspect uremic toxins as the cause of olfactory decline (Raff et al., 2008; Bomback & Raff, 2011). It is thought that p-cresol is the specific uremic toxin to cause a decline in olfactory function because it is not easily filtered out of the blood during dialysis (Meijers et al., 2010). The study sought to set up a control group for future studies by demonstrating p-cresol is not in the blood of healthy participants and to collect normative values for an olfactory threshold test. Results supported the hypothesis that p-cresol is not present in the blood of healthy participants, which strengthens the idea that p-cresol may be the cause of olfactory dysfunction in ESRD patients.

DEDICATION

To my mother, father, and Joe for all of their encouragement and support.

ACKNOWLEDGEMENTS

This project could never happen without the help of many people. First I would like to thank Dr. Nicky Ozbek for all of the time, patience, and advice throughout both my undergraduate and graduate years. You have helped expand my research interests and have given me multiple opportunities that will help in my future goals. I would also like to thank Dr. Manuel Santiago and Dr. Stefanie Whitson for all of their help with the research. I can never begin to repay the time you two have sacrificed to help a student not even in your department. You two have shown me that completely different departments can work together and make a great team.

I would like to thank the olfactory research team: William Tewalt, Carrie LeMay, Hannah Tumlin, Joseph Jones, Matthew McCurdy, Naomi Whitson, Chelsey Mertens, and Eric Peterson. I would also like to thank the chemistry team: Kenneth Hodges, William Tate, Stephanie Harris, Robert Dunn, and Jaslynn Murphy. I would never have been able to complete this project without all of your help.

Finally, I would like to thank Dr. Liz Culler, Whitney Garvich, Craig Glass, and the staff at Blood Assurance for working with us. You were a tremendous help in making this project happen and without your help, I would not have been able to even begin this project. I would also like to thank my friends and family for all of their support and to all of the professors who allowed me to use their classes for my research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CKD, Chronic kidney disease

ESRD, End-stage renal disease

MDD, Major depressive disorder

SGA, Subjective Global Assessment

OBPs, Olfactory binding proteins

WUTC, Wheeler-University of Tennessee at Chattanooga

HPLC, High Performance Liquid Chromatography

CHAPTER I

INTRODUCTION AND BACKGROUND

Studies have examined olfactory identification and detection in special populations including Alzheimer's disease, Parkinson's disease, and kidney disease. These populations have been studied to investigate differences in olfactory function between the healthy population and disease population in question. Additionally, researchers have sought to determine whether different measures of olfactory function can pin point what stage of the disease a person is in, or if different olfactory measures can be used to predict whether individuals might develop a certain disease (Huttenbrink, Hummel, Berg, Gasser, & Hahner, 2013).

The kidney disease population has been studied extensively with researchers investigating both the presence of olfactory identification and detection deficits as well as how they relate to nutrition/health status and timing of dialysis (Griep et al., 1997; Raff et al., 2008). Many of these studies have found evidence supporting the idea that the end-stage renal disease (ESRD) population experiences olfactory deficits in both identification and detection; however, they have not identified when olfactory deficits occur during the progression of the disease or what causes these deficits (Bomback & Raff, 2011).

Background

Chronic Kidney Disease

Stages of the Disease. Chronic kidney disease (CKD) is a condition in which the kidney is either damaged or experiences decreased function for at least three months. A person's kidneys with this disease can no longer clean out toxins and waste from the blood or carry out normal functions such as regulation of blood pressure, water, and important chemicals (Levey et al., 2003). CKD has its etiology based in various health problems, such as high blood pressure, diabetes, chronic kidney infections, and numerous other genetic and external causes. The person's kidneys do not suddenly cease to work, but rather decline in function throughout an extended period of time, as identified by the five stages of CKD (Levey et al., 2003). The fifth stage of CKD is typically referred to as end-stage renal disease (ESRD) and is classified as the person's kidneys only functioning at <15% normal capacity (Levey et al., 2003). However, ESRD is used to indicate that a kidney disease patient is being treated with dialysis or is considered for transplantation, regardless of what stage of CKD the patient is in (Levey et al., 2003). Once a person is given the classification of ESRD, he/she must begin dialysis in order for his/her kidneys to regain some functionality, but remains dependent on the procedure in order live.

Comorbidities and Olfactory Function. ESRD patients do not generally have only ESRD, but instead multiple health problems including diabetes, hypertension, depression, and sleep disorders. These comorbidities have been studied with each other, independent of ESRD,

with olfactory function being one of the most common problems, for example in diabetes and depression. Researchers have recently found that diabetes, specifically the use of insulin, affects olfactory function in adult mice (Aime et al., 2012). Insulin's role in olfactory function has yet to be completely determined, but the researchers of this particular study found that the injection of insulin decreased olfactory detection in the mice. One possible explanation for this phenomenon is insulin's role in eating habits. Insulin interacts with the olfactory bulb in order to let the animal know when it is satiated to prevent over eating (Aime et al., 2012).

One study by Ketterer et al. (2011) examined the role of insulin on olfactory function. All participants fasted ten hours before testing began and some participants volunteered to receive insulin while the control group did not receive insulin. The participants who received insulin had higher olfactory threshold values, indicating higher insulin levels reduce olfactory function. Another study conducted by Stafford and Welbeck (2011) found that participants who were satiated had higher threshold scores compared to those who were not satiated. Because insulin levels increase after eating, the authors suggest that insulin levels are a very plausible factor in olfactory function.

Depression is also linked to olfactory function in many ways, including its interactions with the olfactory bulb as well as other parts of the brain. Several areas of the brain involved in olfactory function processes are used in emotional processes as well. The piriform, orbitofrontal, and insular cortex have been found to be part of human odor perception (Zattore et al., 1997). Zald and Pardo (1997) also found the amygdala is activated when presented with an aversive or novel odor. The orbitofrontal cortex participates in the processing of emotional information (Pardo et al., 1993) and the insula is activated during the experience of negative emotions (Lane

et al., 1997). The amygdala plays a role in encoding positive and negative emotions and has been found to only be activated during negative emotions (c.f. Pause, Miranda, Goder, Aldenhoff, & Ferstl, 2001). It has also been found that there is abnormal activation of the amygdala and orbitofrontal region in depressive patients (Drevets et al., 1992). These findings suggest a possible link between depression and olfactory function.

In one study, (Negoias et al., 2010), olfactory bulb volume was found to be smaller in patients with major depressive disorder (MDD) compared to control groups. Scores on Beck's Depression Inventory were negatively correlated to olfactory bulb volume and olfactory sensitivity. Other studies have found that MDD patients have reduced odor perception (Pause, Miranda, Goder, Aldenhoff, & Ferstl, 2001) and decreased odor identification (Gross-Isseroff et al., 1994; Serby, Larson, & Kalkstein, 1990), further supporting the idea that olfactory function is correlated with depression. Complete loss of olfactory function or distortion of perceived smells has been studied in relation to depression. Smeets et al. (2009) found that patients who experienced parosmia (experiencing a different perceived odor from what the actual smell is) as well as anosmic patients were more likely to experience depression. Even though depression has been predominantly correlated with odor detection and identification, perceived pleasantness or liking of an odor has been shown to change with the presence of depression (Satoh et al., 1996). This may be caused by alterations in brain structures, such as the amygdala, that processes pleasant and unpleasant emotions and perceives aversive or novel odors. Changes in the amygdala due to depression may cause the perception of an odor to a patient to change and be experienced differently from a person with a normal amygdala.

These studies point to the possibility that comorbidities cause olfactory dysfunction in ESRD patients as the rates of depression and diabetes are higher in ESRD patients. The prevalence of depression in the ESRD population is thought to range from 20-30% (Chilcot, Wellsted, Silva-Gane, & Farrington, 2008; Kimmel, Cukor, Cohen, & Peterson, 2007) while the rate of diabetes is 35-55% in the ESRD population (U.S. Renal Data System). Because most ESRD patients have one of these comorbidities, there is a possibility that olfactory dysfunction is caused by an interaction of the different diseases.

Olfactory Function in ESRD. Griep et al. (1997), while studying ESRD patients, wrote that odor perception is one of the most important aspects of life. Odor perception leads to appreciation of different types of food, detection of possibly dangerous elements, and overall quality of life. Thus, impaired olfaction sensitivity represents a significant loss.

One of the first studies of impaired olfactory function in the ESRD population was conducted by Schiffman, Nash, and Dackis (1978). They found that compared to a young normal control group, chronic renal failure patients had decreased odor discrimination and rated odors as more unpleasant. Possible reasons for impaired olfactory function according to these authors include: 1) the removal of nutrients through dialysis may inhibit the quick regeneration of cells in the olfactory epithelium 2) Vitamin B_{12} deficiency and peripheral neuropathy may slow down nerve conduction velocities and 3) psychological defense mechanisms may decrease the ability to discriminate odors among the ESRD population (Conrad et al., 1987; Griep et al., 1997). In a subsequent study, Corwin (1989) confirmed that ESRD patients' odor discrimination was impaired compared to control groups. In addition, Corwin (1989) found ESRD patient's odor

discrimination scores to be lower after treatment with dialysis. These findings suggest a fourth theory against the argument that impaired olfactory function is caused by a buildup of uremic toxins. Other studies have found olfactory identification ability to be lower in ESRD patients and to fall by an estimate of 5% between pre and post-dialysis, further enforcing the idea that olfactory function impairment is not due to uremic toxin buildup given that dialysis does not help (Conrad et al., 1987).

On the other hand, Griep et al. (1997) found a positive correlation between creatinine clearance and smell perception, indicating that olfactory function may be tied to uremic toxin levels. However, the correlation between creatinine clearance and smell perception was found in patients not undergoing dialysis. This finding is similar to Corwin's in that dialysis was not responsible for improvement in olfactory function. One explanation is that it may take days for olfactory function to become normal after urea concentrations are lowered (Griep et al., 1997). Another explanation is that the olfactory system has a lower threshold for uremic toxins and thus cannot be completely repaired but rather only slightly improved because the levels of uremia are above its threshold (Owen, Lew, Liu, Lowrie, & Lazarus, 1993). Further findings from Griep et al.'s (1997) study demonstrated a negative correlation between both uremia and serum phosphorus and smell perception, supporting the theory that uremic toxins may play a role in olfactory function impairment in this group. The uremic toxin argument is further supported by the complete recovery of olfactory function in kidney transplant patients, indicating that the complete clearance of the uremic toxins allowed for olfactory function to return (Griep et al., 1997). Raff et al. (2008) also found no correlation between retained uremic molecules and

olfactory function, but speculated that there may be other small uremic toxins in play and that the relationship between impaired olfactory function and uremic molecules is complex.

Malnutrition has become a topic of interest among the ESRD population because it affects up to 75% of ESRD patients (Raff et al., 2008). Several factors contribute to the development of malnutrition, including poor oral hygiene, gastrointestinal complications of comorbid diseases, increased levels of inflammatory cytokines, changes in tryptophan, serotonin, ghrelin, and leptin levels, and increased levels of middle molecules and indoles (uremic toxins) (Raff et al., 2008). Recently malnutrition has been related to olfactory function impairment in the ESRD population. Raff et al. (2008) compared the olfactory function of 31 hemodialysis patients to their nutritional scores determined by the Subjective Global Assessment (SGA) score and retained uremic molecules. They found that lower SGA scores were inversely correlated with smell scores from the University of Pennsylvania Smell Identification Test (UPSIT) (Doty, Shaman, Kimmelman, & Dann, 1984), that the healthiest of the ESRD group was comparable to the control group's scores, but the ESRD patients with lower SGA scores scored lower on the UPSIT, showing that nutritional status relates to olfaction. Thus, a fifth theory explaining olfactory sensitivity impairment in ESRD patients is lack of essential nutrients to build proteins, in other words, malnutrition.

Measurement. Measurement of olfactory sensitivity in ESRD patients may be a factor influencing the differing outcomes. Olfactory identification relies more on verbal labeling than olfactory sensitivity. Although the participant may be able to smell the odorant, they may not be able to put a name to it, leading the participant to guess. If the participant guesses wrong on an

identification test, it indicates that their olfactory function is low compared to the normative values. This may skew results in multiple ways. First, children do not have large vocabularies and may not be able to name certain odorants; thus their odor processing abilities may be underestimated. Also, women tend to outperform men on olfactory identification tests (Doty & Cameron, 2009). This may be due to verbal memory rather than actual olfactory function. Threshold tests can control for verbal memory because they do not rely on the participant to label an odorant, but rather tell whether or not an odorant is detected. By using threshold tests, researchers can also obtain the specific concentration at which someone can detect an odorant, compared to an identification test where it is all or nothing.

Children who were diagnosed with chronic kidney disease did not score differently on an olfactory identification test from the control group and healthy group, indicating that olfactory function did not relate to renal failure (Armstrong, Laing, Wilkes, & Kainer, 2010). However, the researchers stated that the outcome may have been different if they tested detection thresholds or intensity instead of only identification.

Landis et al. (2011), using both a threshold test containing n-butanol and acetic acid and Sniffin' Sticks, an identification test, measured olfactory function of dialysis patients before and after dialysis. Compared to control groups, olfactory threshold and identification were worse in dialysis patients. When threshold scores and identification scores were examined before and after an hour of dialysis, it was found that overall scores improved. Identification scores of the dialysis patients were similar to the control group's scores. However, the acetic acid threshold values, although improved after dialysis, were not comparable to the control group scores, and the threshold values for n-butanol remained unchanged before and after dialysis. Given the

contradictory information about the role of uremic toxins on olfaction in the ESRD population, the authors suggest that more than one dialysis treatment is needed in order to improve olfactory function.

Role of Uremic Toxins. There are many uremic toxins that play a role in ESRD including p-cresol, creatinine, and urea (Vanholder et al., 2003). Uremic toxins are chemicals normally filtered out by healthy kidneys, but once the kidneys decline in function, the toxins build up in the body and interact negatively with biological functions of the body (Vanholder et al., 2003). Uremic toxins have been examined for their effects on mortality rates and nutrition, but not on their effects in olfactory function (Campbell, Bauer, & Johnson, 2012; Meijers et al., 2010; Raff et al., 2008).

Researchers began focusing on p-cresol due to its relationship with mortality rates in the ESRD population as well as the poor clearance rate of the toxin (Lin et al., 2011; Meijers et al., 2010). In one study, researchers found evidence of p-cresol binding to multiple proteins, leading to the prevention of other vital molecules or even medications binding to proteins. It was also found that p-cresol inhibited the production of leukocytes which led to decreased infection prevention (De Smet, 2003). Multiple studies have confirmed that p-cresol binds to proteins and comes in two forms: free and bound, indicating whether or not it is bound to a protein. The chemical name and molecular weight changes between the two forms, but there has been a debate about whether or not there is a difference in the clearance rate during dialysis between the two. Niwa (1993) found that both forms of p-cresol are harder to clear and reduce in dialysis patients. Other studies have confirmed this finding, suggesting that p-cresol is harder to clear in

general because of the kidney's inability to separate p-cresol from the proteins (Dobre, Meyer, Hostetter, 2013).

Since low molecular weight is the possible cause for decreased filtration rate of p-cresol, studies have begun focusing on different techniques for filtering out the toxin. Lessafer et al. (2000) looked at high and low flux filters to examine if there would be a difference in filtration rate of p-cresol. Although the researchers assumed there would be a difference between the filters since each one is used for specific sizes of uremic toxins, no difference was found (Lessafer et al., 2000). Two studies focused on different types of dialysis and their effects on filtration of p-cresol. The results indicated p-cresol was cleared out significantly better with hemodialysis versus peritoneal dialysis, but the researchers still noted that p-cresol is not cleared out nearly as well as other uremic toxins (Karkar, 2012; Pham et al., 2008). Kreiter et al. (2009) examined the differences in clearance rate using two filters as well and found no difference. However, they also examined the clearance rate of p-cresol after dialysis and filtration and noticed that despite a marked improvement, these processes did not even reach 50% clearance rate of p-cresol. Multiple studies have confirmed these results and authors are beginning to agree that p-cresol is very difficult to clear out of the kidneys efficiently and a better technique for the removal of the toxins needs to be developed (Davenport, 2011; Lee et al., 2010; Martinez et al., 2006).

Although debate continues about how to better clear p-cresol from ESRD patients' kidneys, one study has focused on a different approach to the problem. Nakabayashi et al. (2011) examined ways to treat intestinal problems within the ESRD population. The authors hypothesized that phenol levels and p-cresol levels in the body affected bowel movements of the

patients and that one way to treat this problem would be through the use of synbiotics (Nakabayashi et al., 2011). Although the sample size was small (N=7), they found that the use of synbiotics not only improved bowel movements of the patients, but also decreased the serum p-cresol level. This finding suggests that there may be a solution to the build-up of p-cresol in ESRD patients that can be paired with dialysis in order to improve overall kidney health.

The majority of studies involving p-cresol in the ESRD population have focused on the levels of p-cresol present in either free or bound form as well as the filters that are used during dialysis. Although these studies have provided an abundance of information that can be used to improve upon the filters currently being used, not many studies are focusing on how to reduce the level of p-cresol in the body by other means, with the exception of the study of synbiotics (Nakabayashi et al., 2011).

Olfactory Binding Proteins

Olfactory binding proteins (OBPs) are low molecular weight proteins that are found in nasal mucus of various species (Pelosi, 1994; Pevsner, Hou, Snowman, & Snyder, 1990). Although advances have been made about the functions of OBPs, the function of these proteins is still not completely known. Many studies have focused predominately on insect OBPs, but there are studies beginning to investigate the role of these proteins in mammals, a few including humans (Steinbrecht, 1996). In humans, there is evidence of two different types of OBPs: OBP-A and OBP-B (Briand et al., 2002). The roles of these specific OBPs are not completely understood, but studies suggest that they carry odorants, specifically lipophilic odorants, across

the epithelium in order to be detected by olfactory receptor neurons (Briand et al., 2002; Pevsner, Hou, Snowman, & Snyder, 1990).

Bomback and Raff (2011) have suggested that uremic toxins may play a role in damaging the olfactory epithelium (which could play a role with OBPs) and other higher order olfactory structures, causing olfactory dysfunction. These results further add to the hypothesis that olfactory dysfunction is directly related to kidney disease.

Purpose of the Study

As the cause of olfactory dysfunction in the ESRD population remains unknown, this pilot study was designed to set up a control group for future olfaction studies in the ESRD population. Raff et al. (2008) and Bomback and Raff (2011) along with many other researchers have suggested the possibility of uremic toxins playing a role in olfactory dysfunction. As p-cresol is one of the most noted of the unfiltered toxins, it made sense to examine it.

One of the first goals is to determine whether or not p-cresol is present in general, healthy people. Since p-cresol is only studied in relation to ESRD, not many studies explicitly state whether or not p-cresol might still be present in healthy people, just in lower amounts. In order to determine if p-cresol is the cause of olfactory dysfunction, it must be shown that healthy people do not have it. If healthy people with normal olfactory function also have p-cresol, then other factors in addition to the amount of p-cresol might be at play in the ESRD population. It is hypothesized that healthy people will not have p-cresol in their blood.

The second goal is to determine threshold values of the healthy population using a new threshold test. Because the one commercially available threshold test does not include an odorant

for p-cresol nor for vanillin, which shares a similar chemical structure, a new threshold test and procedure, the Wheeler-University of Tennessee at Chattanooga (WUTC) threshold test (Tewalt, 2012) was developed. ESRD patients should not even be able to detect this p-cresol because it is already present in their system. It is hypothesized that p-cresol may be taken up by OBP-As in humans for transport to the olfactory epithelium. If this is the case, an odorant similar to p-cresol will be transported in the same manner. Vanillin is similar to p-cresol in that it is hydrophobic, has a benzene ring, and is a very detectable odorant at high concentrations, hence its selection as one of the test odorants (Appendix D). Thus, the second hypothesis is that threshold values for p-cresol and vanillin will be similar, thus having a positive correlation. These values will then be used as a base of comparison for the ESRD population.

Given that p-cresol is thought to take up OBPs, and molecules similar to it would be transported in the same way, p-cresol may block the detection of vanillin. If both p-cresol and vanillin use the same OBPs, the detection of one odorant may use all of the OBPs, leaving no more for the detection of the second odorant. The third hypothesis is that smelling p-cresol will block the detection of vanillin.

Finally, the fourth hypothesis is that there will be no correlation between p-cresol and threshold values. Even though p-cresol is hypothesized to influence olfactory function, healthy people should not have p-cresol in their blood. Because it is also hypothesized that healthy participants' blood samples will not contain p-cresol, it follows that no correlation will be found.

CHAPTER II

METHODS

Participants

The sample consisted of 15 students from The University of Tennessee at Chattanooga. Ten participants (67%) identified as Caucasian, 4 (27%) as African-American, and 1 (6%) as Bi-racial. Participants included 7 females and 8 males with age of participant ranging from 18-24. The mean age of the participants was 19.86 (*SD*=1.66). Out of the participants, one was currently smoking and four had smoked previously, but not currently. Participants were recruited from introductory psychology courses as well as upper level classes in psychology and chemistry, and were given extra credit at the discretion of the instructor for their participation. Although multiple medical disorders and medications were listed on the demographics questionnaire, only a few were present in this sample: one participant had thyroid disorder, five had asthma, two had lung trouble, one had a broken nose, one had anemia, two had a history of concussions, two had food allergies, four had sinus problems, ten had seasonal allergies, two had medical allergies. Participants taking medications were as follows: two taking anti-inflammatories, one taking antihistamines, and one taking stimulants.

Measures

Blood Analysis. Students from the Chemistry department at UTC were recruited in order to analyze the blood samples, per IRB and Blood Assurance regulations. The instruments used to analyze the blood samples included a Centrifree Micropartition to centrifuge samples, a ThermoFinnigam UV1000 SpectraSystem High Performance Liquid Chromatography (HPLC), columns for the HPLC, and a ThermoFinnegan Polaris Q Mass Spectrometer equipped with a Direct Probe Controller (De Smet et al., 1998).

Demographics Questionnaire. A demographics questionnaire was used to collect medical information from the participants. The questionnaire asked for age, ethnicity, gender, and level in college of the participants. Questions also included how often, if ever, the participants engaged in smoking and whether or not participants had a medical condition listed on the questionnaire (see Appendix C).

Wheeler University of Tennessee at Chattanooga Threshold Test. The WUTC (Tewalt, 2012) was used in order to measure the participants' olfactory sensitivity. The test consists of five odorants including vanillin, pinene, ethanol, isoamyl acetate (banana), and p-cresol. The test is double-blinded and randomized in order to prevent researcher bias as well as desensitization to the odorants. Along with randomization, each odor is presented twice throughout the test in order to obtain reliability/internal consistency. Blanks are also used in order to obtain a measure of false positives because false positives can be correlated to sensitivity as well as possible exclusion criteria.

Vanillin and P-cresol Tubes. Two tubes were used to present vanillin and p-cresol to the participants in order to investigate the possibility of p-cresol preventing vanillin from being detected. P-cresol was always presented first followed by vanillin. Both tubes were equal in concentration in order to control for intensity. The answer choices for participants to choose from in order to identify the odor in the second tube were as follows: peanut, lavender, vanilla, and black pepper, with vanilla being the correct answer.

Procedures

Protection of Health Information. Before beginning the project, all researchers involved in the project were required to take the CITI and NIH certification tests. These tests involve HIPPA training and researchers who take these tests in turn become HIPPA certified. With these certifications, the researchers were able to handle the health information on the participants' demographic questionnaires.

Blood Samples at Blood Assurance. Blood Assurance of Chattanooga agreed to draw 10 mL of un-centrifuged blood from participants of the study as long as the participants donated blood during the process. Specific dates were chosen in coordination with representatives at Blood Assurance for participants to donate blood and the extra 10 mL vial. Participants were then instructed via their professors to go to Blood Assurance on the designated dates to donate blood and the additional 10 mL vial.

Researchers on the olfactory research team set up a table at Blood Assurance for participants to sign in and read over the informed consent form (Appendix A). While going over

the informed consent form, the participants also signed up for a time to participate in the threshold test. The researchers explained that the participants' names would not be put onto the blood vials. A sticker with a serial number was placed on both the blood vial and the participants' informed consent forms in order to link the threshold test results to the blood samples. After the threshold test and blood sample data were put together, the sticker on the informed consent form was destroyed in order to protect confidentiality. After participants were given a vial, participants were then instructed to go to the front desk and sign in for a normal blood donation. The participants were taken to a private room to answer questions and undergo a screening process, per Blood Assurance procedures. During the blood donation, the nurses drew the extra vial of blood for the study and placed the vials in an insulated, properly marked box. At the end of the day, the blood was stored in a refrigerator set to 4 degrees Celsius in the biochemistry lab at the Chemistry Department for analysis.

Blood Analysis. The blood analysis occurred in several steps. The first step was centrifuging one milliliter of each sample in order to run in the HPLC. The samples were first dissolved in HPLC-water before being run in the HPLC. Once the columns used in the HPLC were cleaned for five minutes using methanol, the samples were run for 18 minutes in order to separate out different samples for further testing. Once the samples were filtered out, they were run in a Mass Spectrometer in order to determine the molecules present in the samples. Researchers then examined mass spectrum results in order to determine the molecules present in the samples. Specific details of the procedures used for the blood analysis can be found in Appendix E written by one of the principal investigators, Dr. Santiago.

Threshold Test. Participants arrived at the administration room the day they indicated on the sign-up sheet that was provided at Blood Assurance. One of the two assigned researchers went over the informed (Appendix B) consent with the participant. Once the participant completed the demographics questionnaire (Appendix C), the researcher began administering the threshold test.

Two researchers were in charge of administering the test. In order for the test to be double-blind, the first researcher handed the tube to the second researcher. The second researcher was in charge of holding the tube under the participant's nose for three seconds. Once the participant responded "yes" or "no" to whether or not they detected an odor from the tube, the first researcher recorded the response and handed the second researcher another tube. Each tube was assigned a number and the order of presentation was generated by a computer and printed for the first researcher to follow.

In order to prevent contamination of the test with external odorants, the researchers wore latex free, powder free gloves. The researchers also kept all odorous objects (e.g., food) out of the testing room at least one hour prior to testing time.

Vanillin and P-cresol. Once the participants completed the threshold test, they were asked to complete the final part of the threshold test, which including smelling two tubes. The researcher told the participant that two tubes would be presented, one immediately after the other, and to indicate on a sheet "yes" or "no" if they detected an odor in the second tube, and if so, to identify the odor by picking one of the four choices provided. The researcher then held the first tube (p-cresol) under the participant's nose for five seconds and immediately switched the

tubes and presented the second tube (vanillin) for five seconds. Vanillin was never presented first due to difficulty in labeling/describing p-cresol on the multiple choice answers. Participants then circled “yes” or “no” on the sheet and picked an answer, from four choices, on the sheet regarding the odor of the second tube. The four choices were presented in random order for each participant and included Black Pepper, Peanut, Lavender, and Vanilla, based on answer choices provided in the UPSIT.

CHAPTER III

RESULTS

Blood Analysis

In order to determine the presence of p-cresol, mass spectrometry was performed for each sample collected from the HPLC. Molecular weights on the mass spectra were examined for each blood sample at different times within two minutes to determine which molecules were present. Molecular weights up to 300 amu were examined (Figure 1). The data showed that possible remnants of p-cresol were present in the samples, but at extremely low quantities. Other amino acids that have similar molecular structures to p-cresol were present in higher quantities: tryptophan, tyrosine, and phenylalanine.

Because there were only possible remnants of p-cresol, and not positive identifications of the molecule, it was determined that p-cresol was not present in the blood. This conclusion was further strengthened by the fact that these remnants were in extremely low quantities.

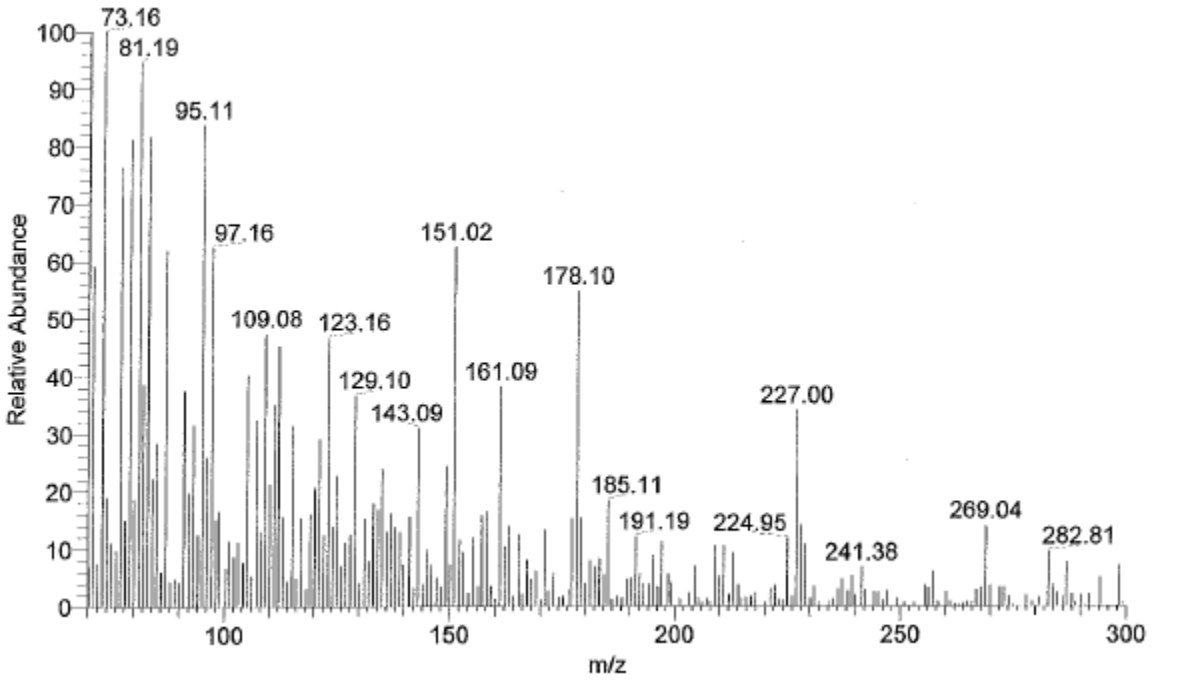


Figure 3.1 Example of Mass Spectrum Data

Threshold Test

In order to obtain participants' threshold values, logistic regression was used. This type of analysis was chosen due to the nature of the threshold test. Because the test was randomized and each tube presented twice, the logistic regression allowed for both presentations to be taken into account. The thresholds for each participant varied between odorants (Table 1). The scores are interpreted as follows: the higher the threshold value, the worse the participant's sensitivity to that odor. However, the threshold values represent predicted threshold values, which are the predicted concentrations of the odorant the participant would be able to detect. Multiple interactions were examined using the threshold values. Reliability estimates were not run due to the small sample size.

Gender and Ethnicity. Relations between gender and threshold values as well as ethnicity and threshold values were examined. In order to examine this and other analyses involving the threshold values, simple regressions were used rather than t-tests due to the extreme variation in threshold values. There were no significant interactions between threshold values and gender or threshold values and ethnicity.

Medical Conditions. Simple regressions were run to examine relationships between medical history and threshold values. Only medical histories that had three participants in the category were examined due to such a small sample size: asthma, seasonal allergies, and sinus problems. There were no significant relations for each medical condition when examined with each separate odorant on the threshold test. When controlling for all three variables, no significant relationships with the threshold values were found.

Threshold Value Relationships. In order to test the hypothesis involving the relationship between threshold values for p-cresol and vanillin, Pearson r correlations were used. The results for the correlation between threshold values for vanillin and p-cresol were not significant. The relationship between threshold values for vanillin and ethanol was significant (r=0.887, p<0.001). The relationship between threshold values for isoamyl acetate and how many blanks were hit was also significant (r=-0.606, p=0.017). All other correlations between threshold values were not significant.

Vanillin and P-cresol Tubes

One participant was excluded from the results of the tube presentations due to a peanut allergy. Although peanut was not presented, it was one of the answer choices. Out of the remaining 14 participants, only one was not able to smell vanillin.

Table 3.1 Threshold Values for Participants

Participant	Vanillin	Pinene	Ethanol	Isoamyl Acetate	P-cresol	Blanks
1	.00	187.93	563.16	11219.73	1415.32	0
2	10.68	125.14	.00	1384.43	240.13	9
3	14.60	.04	6008.61	3978.87	.97	9
4	15.00	58.37	323196.34	3753.29	33.77	7
5	15.83	81.28	.00	1384.43	73.91	7
6	44.19	124.86	211.70	5529.37	59.61	6
7	63.80	4977.02	28.47	11048.20	1429.61	1
8	65.20	357.57	604.02	5529.37	706.86	4
9	88.49	150.48	38.01	5445.13	706.86	0
10	114.60	250.03	26594.80	2766.57	706.86	2
11	176.49	179.53	17.06	7815.99	706.86	0
12	178.27	362.45	1.10	5516.61	7396.55	0
13	337.74	457.94	.71	11049.25	2172.85	0
14	352.14	277.97	19.54	5529.37	499.59	2
15	3460.11	14.90	715024.61	1888.10	348.11	1

Note: Thresholds for each participant are ranked lowest to highest based on vanillin

CHAPTER IV

DISCUSSION

The purpose of the study was to investigate the presence of p-cresol in the healthy population and to examine the relationship of p-cresol to olfactory threshold values. As hypothesized, no p-cresol was detected in the blood of the participants. Because this hypothesis was supported, the fourth hypothesis was supported as well. Given that there was an extremely low amount of p-cresol present in the blood of the participants, if any, no correlations with the threshold test could exist. The molecules tryptophan, tyrosine, and phenylalanine were all found in the blood samples of the participants. All of these molecules share the characteristic of a benzene ring with p-cresol. These molecules may play a role in metabolic mechanisms that form uremic toxins.

Vanillin and p-cresol thresholds were not found to be significantly related. One explanation for this finding is that vanillin and p-cresol are not similar enough. Even though they are both hydrophobic and share the characteristic of a benzene ring, they differ in molecular weight and chemical formula. Other reasons include familiarity and hedonic qualities of the odorants. The smell of vanilla is one of the most common odors to which people are exposed. The odor is typically described as sweet and pleasant which could possibly influence its detection. Because the participants are familiar with the odor, it may be easier to detect. P-cresol is the opposite, often described by the participants as unfamiliar. If the participants are unfamiliar

with the odor, they may have detected the lower concentrations of p-cresol in the tubes during the threshold test, but doubted themselves because they were not familiar with it.

The third hypothesis also was not supported. One possible explanation would be the presentation of the two tubes. The interval between the presentations of the two tubes could have been too long, which could be corrected by using a machine rather than manual presentation. Also, some participants attempted to pull away from smelling the p-cresol tube due to its unpleasant odor. This may have caused the participants to inhale very little p-cresol, increasing the certainty that they would detect vanillin. Another possibility is that p-cresol and vanillin do not use the same OBPs for transport. There may be different OBPs that are specific towards certain odorants and the two odorants do not use the same ones, relating back to the previous argument that they are not completely similar molecules.

One interesting finding for the threshold test was the lack of significant gender differences in threshold values. As noted by Doty and Cameron (2009), previous research has found gender differences in olfactory function. Women have performed better than men, especially at older ages, on olfactory identification tests (Doty & Cameron, 2009). There were no differences in gender performance in this study, possibly due to the fact that it is a sensitivity rather than identification test. Women may perform better than men on identification tests due to a better verbal memory rather than actual odor memory. Another reason there may have been no significant interaction of gender on threshold values would be due to age. Women and men perform roughly the same on olfactory identification tests at younger ages. The difference between the genders is not drastic until around the mid-30s to 40s (Doty & Cameron, 2009). In

addition to these reasons, the small sample size may have prevented any significant differences from being detected.

Although the study focused predominately on vanillin and p-cresol, the results showed a positive correlation between vanillin and ethanol threshold values. Although the relationship is positive, ethanol threshold values were very sporadic. One possibility for this wide distribution of scores would be due to ethanol being hydrophilic. Theoretically, hydrophilic molecules would use a different mechanism of transport to the olfactory epithelium rather than OBPs which transport hydrophobic molecules. This different mechanism of transport may contribute to the wide range of values. Because there is a wide range of scores, caution must be taken when interpreting the relationship between vanillin and ethanol scores.

Another interesting finding was the relationship between isoamyl acetate and the number of blanks. Since the relationship was negative, it would indicate that the higher the threshold score for isoamyl acetate (worse sensitivity), the fewer blanks a participant would hit. This would indicate that the participant who cannot detect banana would do better on the threshold test because he/she would not respond with a "yes" in tubes that contained no odorant. This finding does not make sense in that participants who had lower thresholds, and thus better ability to smell, would be thought to also respond correctly at responding "no" to tubes with no odorants. The only way this result would make sense is if the person were responding with certainty rather than guessing. In this case, blanks may represent a measure of response bias. This finding is contrary to expected results and thus needs to be further investigated in future studies.

Additional results from the threshold test were also interesting. Seasonal allergies, asthma, and sinus problems have the potential of influencing olfactory function. Significant interactions were expected just based on this idea, but none were found. The small sample size could have accounted for non-significance. Future studies need to include more people with medical conditions such as seasonal allergies, asthma, and sinus problems to get a definitive result of the effect of medical conditions on threshold values.

Limitations and Directions for Future Research

This pilot study is limited in several ways. The first limitation is the small sample size. Only 15 participants were recruited, and thus the study is not generalizable. The nature of the study made it difficult to recruit participants. In order to donate blood, participants were told what days they were able to donate, and had the option to go to Blood Assurance from 9 A.M. to 5 P.M. Around 70 participants completed the blood donation portion of the study. The limiting factor was the threshold test. Although participants were able to choose dates and times on a sign-up sheet to participate in the threshold test, they did not always show up. This could have been a result of the participants forgetting the date and time they signed up for, or not wanting to participate any further due to the extra time commitment. Also, the threshold test itself took 45 minutes, limiting the number of times offered throughout the day for participants to sign-up. Only six slots were open on any day and combined with participants not showing up, roughly five people a week would participate. If the threshold test was offered at any time throughout the day, the sample size could have been larger.

The small sample size also limited the ability to obtain powerful statistical results. There were no significant interactions between any of the medical conditions and threshold values.

Future studies should attempt to recruit more participants and, if possible, people specifically in those medical categories to look at the differences in olfactory sensitivity. Future studies can also examine the specific relationship of seasonal allergies to threshold values by determining the pollen count in the area the study is conducted.

Another limitation was the shelf life of the threshold test. After the fifteenth participant, the researchers noticed that the odorants in some of the tubes were beginning to fade, throwing off the concentrations. This was not expected to occur since the pilot threshold test did not fade as quickly. One reason this may have occurred would be the continuous use of the test throughout the day as well as the double presentation of each tube. This would cause the tubes to be open for a longer period of time, exposing the odors to external contaminants for longer periods of time. A few participants would also move their nose towards the tubes and breathe into the tubes. This could affect the tubes by exposing contaminants and distorting the odorants. Future studies should take this into account when deciding how often to remake the threshold test.

In addition to the threshold test, there were limitations in the presentation of the vanillin and p-cresol tubes. The first limitation was the delay between the presentation of p-cresol and vanillin. To date, there have been no studies investigating the possible ability of p-cresol to block detection of vanillin; thus there are no validated procedures. Although the researchers attempted to switch the presentation of the tubes as quickly as possible, there is no way of telling if the p-cresol had already been transported to the epithelium. Also, it was difficult for the researcher to exactly record the time it took to switch the p-cresol and vanillin tubes, so replication of this portion of the study would be difficult.

Future studies need to present both vanillin and p-cresol first. Throughout the study, p-cresol was always the first tube presented followed by vanillin. In order to support the idea that p-cresol blocks odorants, vanillin would also have to be presented first. P-cresol is not a common odorant and does not have a specific smell that people would be able to easily identify. Because it was difficult to give p-cresol another name which people would recognize, it was not presented second. Future studies may want to conduct studies on just naming p-cresol in order to get a commonly agreed upon descriptive label.

In addition to vanillin, other odorants need to be used. Although vanillin is similar in structure to p-cresol, it is not similar in molecular weight. Odorants that are similar to p-cresol in weight need to be investigated. Also, odorants that are hydrophilic need to be used in future studies in order to provide support that p-cresol takes up OBPs. If p-cresol also blocks the detection of hydrophilic odorants, OBPs may not be the only structures of olfaction that p-cresol affects.

Another limitation of the study was the blood analysis. The researchers only investigated the presence of free p-cresol and not protein bound p-cresol. Studies involving p-cresol in the ESRD population look for both types of p-cresol. Although the absence of free p-cresol would indicate that bound p-cresol is unlikely present in the blood of healthy participants, it still should not be ruled out. Because researchers are not sure if bound or free p-cresol is the cause of olfactory dysfunction in the ESRD population, both types of p-cresol should be examined.

Although depression is a comorbidity in the ESRD population, a limitation of this study was that no depression measure was used. Since depression has been studied in relation to olfaction, futures studies need to include a depression measure. Future studies also need to

examine the relationship between the number of comorbidities a person may have and scores on a threshold test because uremic toxins may also play a role in comorbidities in the ESRD population, especially diabetes and depression which have both been investigated with olfaction.

Hunger also needs to be taken into account in future studies. Because insulin levels have been tied to olfactory function, a question regarding the last time a participant ate can be added to the demographics questionnaire.

Conclusion

This pilot study is the first to date to examine the relationship of p-cresol to olfactory function in healthy participants. Although there are many limitations to the study, there are also strengths. First, the study involved the interdisciplinary effort of the psychology and chemistry department. Prior studies involving the ESRD population have not combined the two disciplines. Future studies will benefit from increased interdisciplinary efforts in order to explore topics that are too complex for a single discipline.

Another strength of the study is that it supports the idea that p-cresol is not present in the healthy population. As discussed, the ESRD population suffers from olfactory dysfunction, but few studies have focused on the causes. Even though this pilot study has a small sample size, it is the beginning of multiple studies involving olfaction in the ESRD population. Future studies can replicate the study with larger sample sizes to further confirm the nonexistence of p-cresol in healthy participants, strengthening the idea that p-cresol is the cause of olfactory dysfunction in the ESRD population. Future studies involving olfaction and ESRD need to include p-cresol in olfaction tests in order to strengthen this argument.

REFERENCES

Aime, P., Hegoburu, C., Jaillard, T., Degletagne, C., Garcia, S., Massaoudi, B., … Julliard, A. K. (2012). A physiological increase of insulin in the olfactory bulb decreases detection of a learned aversive odor and abolishes food odor-induced sniffing behavior in rats. *PLoS One, 7*(12), 1-13. doi: 10.1371/journal.pone.0051227

Armstrong, J. E., Laing, D. G., Wilkes, F. J., & Kainer, G. (2010). Smell and taste function in children with chronic kidney disease. *Pediatric Nephrology, 25,* 1497-1504.

Briand, L., Eloit, C., Nespoulous, C., Bezirard, V., Huet, J., Henry, C., … Pernollet, J. (2002). Evidence of odorant-binding protein in the human olfactory mucus: Location, structural characterization, and odorant-binding properties. *Biochemistry, 41,* 7241-7252.

Bomback, A. S. & Raff, A. C. (2011). Olfactory function in dialysis patients: A potential key to understanding the uremic state. *Kidney International, 80,* 803-805. doi: 10.1038/ki2011219

Campbell, K. L., Bauer, J. D., & Johnson, D. W. (2012). Role of nutritional impact symptoms in predicting nutritional status and clinical outcome in hemodialysis patients: A potential screening tool. *Journal of Renal Nutrition*, 1-6. doi: 10.1053/j.jm.2012.07.001

Chilcot, J., Wellsted, D., Silva-Gane, M., & Farrington, K. (2008). Depression on dialysis. *Nephron Clinical Practice, 108,* 256-264.

Conrad, P., Corwin, J., Katz, L., Serby, M., LeFavour, G., & Rotrosen, J. (1987). Olfaction and hemodialysis: baseline and acute treatment decrements. *Nephron, 47,* 115-118.

Corwin, J. (1989). Olfactory identification in hemodialysis: Acute and chronic effects on discrimination and response bias. *Neuropsychologia, 27*(4), 513-522.

Davenport, A. (2011). Role of dialysis technology in the removal of uremic toxins. *Hemodialysis International, 15,* 549-553.

De Smet, R., David, F., Sandra, P., Van Kaer, J., Lesaffer, G., Dhondt, A., Lameire, N., & Vanholder, R. (1998). A sensitive HPLC method for the quantification of free and total p-cresol in patients with chronic renal failure". *Clinica Chimica Acta, 278,* 1-21.

De Smet, R., Van Kaer, J., Van Vlem, B., De Cubber, A., Brunet, P., Lameire, N., & Vanholder, R. (2003). Toxicity of free p-Cresol: A prospective and cross-sectional analysis. *Clinical Chemistry, 49*(3), 470-478.

Dobre, M., Meyer, T. W., & Hostetter, T. H. (2013). Searching for uremic toxins. *Clinical Journal of the American Society of Nephrology, 8*, 322-327. doi: 10.2215/CJN.04260412

Doty, R. L. & Cameron, E. L. (2009). Sex differences and reproductive hormone influences on human odor perception. *Physiological Behavior, 97*(2), 213-228. doi: 10.1016/j.physbeh.2009.02.032

Doty, R. L., Shaman, P., Kimmelman, C.P., & Dann, M. S. (1984). University of Pennsylvania Smell Identification Test: A rapid quantitative olfactory function test for the clinic. *Laryngoscope, 94*(2), 176-178.

Drevets, W. C., Videen, T. O., Price, J. L., Preskorn, S. H., Carmichael, S. T., & Raichle, M. E. (1992). A functional anatomical study of unipolar depression. *The Journal of Neuroscience, 12,* 3628-3641.

Griep, M. I., Van der Niepen, P., Sennesael, J. J., Mets, T. F., Massart, D. L., & Verbeelen, D. L. (1997). Odour perception in chronic renal disease. *Nephrology Dialysis Transplantation, 12*, 2093-2098.

Gross-Isseroff, R., Luca-Haimovici, K., Sasson, Y., Kindler, S., Kotler, M., & Zohar, J. (1994). Olfactory sensitivity in major depressive disorder and obsessive compulsive disorder. *Society of Biological Psychiatry, 35,* 798-802.

Huttenbrink, K., Hummel, T., Berg, D., Gasser, T., Hahner, A. (2013). Olfactory dysfunction: Common in later life and early warning of neurodegenerative disease. *Deutsches Arzteblatt International, 110*(1-2), 1-7.

Karkar, A. (2012). Modalities of hemodialysis: Quality improvement. *Saudi Journal of Kidney Diseases and Transplantation, 23*(6).

Ketterer, C., Heni, M., Thamer, C., Herzberg-Schafer, S. A., Haring, H., & Fritsche, A. (2011). Acute, short-term hyperinsulinemia increases olfactory threshold in healthy subjects. *International Journal of Obesity, 35*, 1135-1138. doi: 10.1038/ijo.2010.251

Kimmel, P.L. & Peterson, R.A. (2006). Depression in patients with end-stage renal disease treated with dialysis: Has the time to treat arrived? *Clinical Journal of the American Society of Nephrology, 1*(3), 349-352.

Kreiter, D. H., Hackl, A., Rodriguez, A., Chenine, L., Moragues, H. L., Lemke, H., … Canaud, B. (2010). Protein-bound uraemic toxin removal in haemodialysis and post-diluation haemodiafiltration. *Nephrology Dialysis Transplantation, 25,* 212-218. doi: 10.1093/ndt/gfp437

Landis, B. N., Marangon, N., Saudan, P., Hugentobler, M., Giger, R., Martin, P., & Lacroix, J. (2011). Olfactory function improves following hemodialysis. *Kidney International, 80,* 886-893. doi: 10.1038/ki.2011.189.

Lee, C., Kuo, Y., Chen, Y., Hsu, C., Lee, W., Tsai, Y., … Chen, J. (2010). Factors associated with blood concentrations of indoxyl sulfate and p-Cresol in patients undergoing peritoneal dialysis. *Peritoneal Dialysis International, 30,* 456-463. doi: 10.3747/pdi.2009.00092

Lessafer, G., De Smet, R., Lameire, N., Dhondt, A., Duym, P., & Vandholder, R. (2000). Intradialytic removal of protein-bound uraemic toxins: Role of solute characteristics and of dialyser membrane. *Nephrology Dialysis Transplantation, 15,* 50-57.

Levey, A. S., Coresh, J., Balk, E., Kausz, A. T., Levin, A., Steffes, M. W., Hogg, R. J., … Eknoyan, G. (2003). National Kidney Foundation practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *Annals of Internal Medicine, 139*(2), 137-147.

Lin, C., Wu, C., Pan, C., Chen, Y., Sun, F., & Chen, H. (2011). Serum concentrations of p-Cresol and indoxyl sulfate in elderly hemodialysis patients. *International Journal of Gerontology, 5,* 80-83. doi: 10.1016/j.ijge.2011.04.010

Martinez, A. W., Recht, N. S., Hostetter, T. H., & Meyer, T. W. (2005). Removal of p-cresol Sulfate by hemodialysis. *Journal of the American Society of Nephrology, 16,* 3430-3436. doi: 10.1681/ASN.2005030310

Meijers, B. K. I., Claes, K., Bammens, B., de Loor, H., Viaene, L., Verbeke, K., … Evenepoel, P. (2010). p-Cresol and cardiovascular risk in mild-to-moderate kidney disease. *Clinical Journal of the American Society of Nephrology, 5,* 1182-1189. doi: 10.2215/CJN.07971109

Nakabayashi, I., Nakamura, M., Kawakami, K., Ohta, T., Kato, I., Uchida, K., & Yoshida, M. (2011). Effects of symbiotic treatment on serum level p-cresol in haemodialysis patients: A preliminary study. *Nephrology Dialysis Transplantation, 26,* 1094-1098. doi: 10.1093/ndt/gfq624

Negoias, S., Croy, I., Gerber, J., Puschmann, S., Petrowski, K., Joraschky, P., & Hummel, T. (2010). Reduced olfactory bulb volume and olfactory sensitivity in patients with acute major depression. *Neuroscience, 169,* 415-421.

Niwa, T. (1993). Phenol and p-Cresol accumulated in uremic serum measured by HPLC with fluorescence detection. *Clinical Chemistry, 39*(1), 106-111.

Owen, W. F., Lew, N. L., Liu, Y., Lowrie, E. G., & Lazarus, J. M. (1993). The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. *New England Journal of Medicine, 329,* 1001-1006.

Pardo, J. V., Pardo, P. J., & Raichle, M. E. (1993). Neural correlates of self-induced dysphoria. *American Journal of Psychiatry, 31,* 713-719.

Pause, B. M., Miranda, A., Goder, R., Aldenhoff, J. B., & Ferstl, R. (2001). Reduced olfactory performance in patients with major depression. *Journal of Psychiatric Research, 35,* 271-277.

Pelosi, P. (1994). Odorant-binding proteins. *Critical Reviews in Biochemistry and Molecular Biology, 29*(3), 199-228.

Pevsner, J., Hou, V., Snowman, A. M. & Snyder, S.H. (1990) Odorant-binding protein: characterization of ligand binding. *Journal of Biological Chemistry, 265,* 1011-1017.

Pham, N. M., Recht, N. S., Hostetter, T. H., & Meyer, T. W. (2008). Removal of the protein-bound solutes indicant and p-cresol sulfate by peritoneal dialysis. *Clinical Journal of the American Society of Nephrology, 3,* 85-90. doi: 10.2215/CJN.02570607

Raff, A. C., Lieu, S., Melamed, M. L., Quan, Z., Ponda, M., Meyer, T. W., & Hostetter, T.H. (2008). Relationship of impaired olfactory function in ESRD to malnutrition and retained uremic molecules. *American Journal of Kidney Diseases, 52*(1), 102-110. doi: 10.1053/j.ajkd.2008.02.301

Satoh, S., Morita, N., Matsuzaki, I., Konishi, T., Nakano, T., Minoshita, S., … Ayabe, S. (1996). Relationship between odor perception and depression in the Japanese elderly. *Psychiatry and Clinical Neurosciences, 50,* 271-275.

Schiffman, S., Nash, M., & Dackis, C. (1978). Reduced olfactory discrimination in patients on chronic hemodialysis. *Physiology and Behavior, 21,* 239-242.

Serby, M., Larson, P., & Kalkstein, D. (1990). Olfactory sense in psychoses. *Biological Psychiatry, 28,* 829-830.

Smeets, M. A. M., Veldhuizen, M. G., Galle, S., Gouweloos, J., de Haan, A. J. A., Vernooij, J., … Kroeze, J. H. A. (2009). Sense of smell disorder and health-related quality of life. *Rehabilitation Psychology, 54*(4), 404-412. doi: 10.1037/a0017502

Stafford, L. D. & Welbeck, K. (2010). Higher hunger stage increases olfactory sensitivity to neutral but not food odors. *Chemical Senses, 36,* 189-198. doi: 10.1093/chemse/bjq114

Steinbrecht, R. A. (1996). Are odorant-binding proteins involved in odorant discrimination? *Chemical Senses, 21,* 719-727.

Tewalt, W. (2012). An improved odorant threshold test. (Unpublished Master's Thesis). University of Tennessee at Chattanooga, Chattanooga, TN.

Vanholder, R., De Smet, R., Glorieux, G., Argiles, A., Baurmeister, I., Brunet, P., … Zidek, W. (2003). Review on uremic toxins: Classification, concentration, and interindividual variability. *Kidney International, 63,* 1934-1943.

Zald, D. H. & Pardo, J. V. (1997). Emotion, olfaction, and the human amygdala: amygdala activation during aversive olfactory stimulation. *Proceedings of the National Academy of Sciences, 94,* 4119-4124.

Zattore, R. J., Jones-Gotman, M., Evans, A. C., & Meyer, E. Functional localization and lateralization of human olfactory cortex. *Nature, 360,* 339-340.

APPENDIX A

INFORMED CONSENT (BLOOD DONATION)

INFORMED CONSENT FORM

Presence of P-cresol in the General Healthy Population

Please read this consent document carefully before you decide to participate in this study. This research has been approved by the University Institutional Review Board.

Purpose of the research study: The purpose is to determine the levels of p-cresol in the blood samples.

What you will be asked to do in the study:

You will be asked to provide a sample of blood that will be stored for later use in the determination of p-cresol levels.

Time required:

~20 minutes

Risks and Benefits:

Donors will be screened for previously diagnosed allergic response to mild volatile chemicals. Subjects will be exposed to hypodermic needle.

Compensation:

No direct compensation will be given.

Confidentiality:

Your identity will be kept confidential to the extent provided by law. Your information will be assigned a code number. The list connecting your name to this number will be kept in a locked file in my research office that only I and my other research team members have access to. When the study is completed and the data have been analyzed, the list will be destroyed. Your name will not be used in any report.

Voluntary participation:

Your participation in this study is completely voluntary. There is no penalty for not participating.

Right to withdraw from the study:

You have the right to withdraw from the study at anytime without consequence.

Whom to contact if you have questions about the study:
Jessica McKinney (344 Holt Hall, 931-801-4544, jessica-mckinney@mocs.utc.edu)

Dr. Manuel F. Santiago (615 McCallie Avenue, 425.5364 and Manuel-Santiago@utc.edu).

Agreement:

I have read the procedure described above. I voluntarily agree to participate in the procedure and I have received a copy of this description.

Participant: _____ Date: _____

If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact Dr. Bart Weathington, Chair of the Institutional Review Board, at 423-425-4289. Additional contact information is available at www.utc.edu/irb

APPENDIX B

INFORMED CONSENT (THRESHOLD TEST)

INFORMED CONSENT FORM (Threshold Test)

Odor Sensitivity and the presence of P-cresol

Please read this consent document carefully before you decide to participate in this study. This research has been approved by the University Institutional Review Board.

Purpose of the research study: The purpose of this study is to measure odor sensitivity in healthy adults and to see if healthy adults are able to identify two odors.

What you will be asked to do in the study:

You will initially be asked to complete a brief demographics page. A researcher will then begin the threshold test by presenting a tube filled with clear liquid beneath your nose for 5 seconds. After these 5 seconds have passed, you will be given 10 seconds to tell the researcher "yes"-you did detect an odor or "no"- you did not detect an odor. The test contains 105 tubes with various odors and concentrations, although not all of the tubes will contain odors. Finally, you will be presented with one scratch and sniff card which you will be asked to scratch and identify the odor and then do the same with a second scratch and sniff card.

Time required:

~50 minutes

Risks and Benefits:

You may experience some nasal dryness from prolonged smelling. We do not anticipate any direct benefit from the study, but we do appreciate your participation as this will add to a growing body of research that will benefit other people in the future.

Compensation:

No direct compensation will be given.

Confidentiality:

Your identity will be kept confidential to the extent provided by law. Your information will be assigned a code number. The list connecting your name to this number will be kept in a locked file in my research office that only I and my other research team members have access to. When the study is completed and the data have been analyzed, the list will be destroyed. Your name will not be used in any report.

Voluntary participation:

Your participation in this study is completely voluntary. There is no penalty for not participating.

Right to withdraw from the study:

You have the right to withdraw from the study at any time without consequence.

Whom to contact if you have questions about the study:
Jessica McKinney (348 Holt Hall, 931-801-4544, jessica-mckinney@mocs.utc.edu)
William Tewalt (344 Holt Hall, wtewalt@gmail.com)
Hannah Tumlin (344 Holt Hall, hannah-tumlin@mocs.utc.edu)
Joseph Jones (344 Holt Hall, joseph-jones@mocs.utc.edu)
Aaron Wamsley (344 Holt Hall, aaron-wamsley@mocs.utc.edu)
Dr. Nicky Ozbek (350 Holt Hall, 425-4285 and Nicky-Ozbek@utc.edu).

Agreement:

I have read the procedure described above. I voluntarily agree to participate in the procedure and I have received a copy of this description.

Participant: _____ Date: _____

Print Name: _____

If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact Dr. Bart Weathington, Chair of the Institutional Review Board, at 423-425-4289. Additional contact information is available at www.utc.edu/irb

APPENDIX C

DEMOGRAPHICS QUESTIONNAIRE

Age (in years): _____

Gender (circle one); Male Female

If female, please answer the questions located on the next page. ***

Ethnicity (circle one): Caucasian African American Asian American Hispanic

Bi-Racial Other (please indicate): _____

Do you currently smoke (circle one): Yes No

If yes; How many cigarettes per day?_____; Cigars per day?____

What type of cigarettes do you smoke? _____

How many years have you smoked? _____

If not currently smoking, have you ever smoked? (Circle one): Yes No

If yes, how long ago did you stop?_____

How many cigarettes did you smoke per day?_____; Cigars per day?_____

Did your ability to smell change after you stopped smoking? (Circle one): Yes No

If yes; How?

_____

_____

What is your occupation: _____

Highest grade completed? (Circle only one number):

6 7 8 9 10 11 12 College 1 2 3 4 5 +

Please indicate if you have had past history of the following medical Illnesses. (Circle Yes or No):

High blood pressure	Yes	No	Diabetes	Yes	No
Arthritis	Yes	No	Heart disease	Yes	No
Thyroid disorder	Yes	No	Headaches	Yes	No
Lung trouble	Yes	No	Gout	Yes	No
Epilepsy	Yes	No	Circulation problems	Yes	No
Broken nose	Yes	No	Anemia	Yes	No
Strokes	Yes	No	Eye problems	Yes	No

Asthma..... Yes No Cancer..... Yes No

Please indicate if you have had past history of the following medical Illnesses. (Circle Yes or No):

Hepatitis..... Yes No Ulcer..... Yes No

Hiatal hernia..... Yes No Kidney disease..... Yes No

Pelvic disease..... Yes No Skin disease..... Yes No

Prostate problems..... Yes No Infections..... Yes No

Bleeding/clotting disorder..... Yes No HIV..... Yes No

TB..... Yes No Neurological disease..... Yes No

Deviated septum..... Yes No Sinus problems..... Yes No

Concussion/head trauma..... Yes No Medical allergies..... Yes No

Food allergies..... Yes No Seasonal allergies..... Yes No

Other: _____

Please indicate if you are currently taking any of the following types of medications. (Circle Yes or No):

Antibiotics..... Yes No Antidepressants ..... Yes No

Hormone replacements..... Yes No Antihistamines..... Yes No

Antihypertensive..... Yes No Antianxiety..... Yes No

Lithium ..... Yes No

Anti-inflammatory[†]..... Yes No

[†]Including ibuprofen

Antineoplastic[††]..... Yes No

[††]Examples of Antineoplastics are *Elspar* (asparaginase), *Alkeran* (melphalan), floxuridine, lomustine, procarbazine, thioguanine, thiotepa

Stimulant medications[†††]..... Yes No

[†††]Examples of Stimulant medications are *Adderall* and *Vyvanse*

Have you ever been diagnosed with Sleep Apnea? (Circle one):..... Yes No

***Females (optional, But VERY BENEFICIAL to answering research questions)**

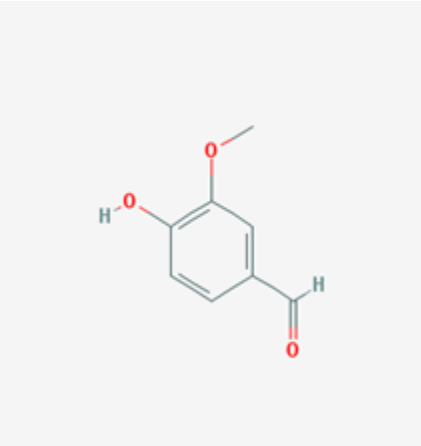
If **FEMALE**; Are you currently on your menstrual cycle? (Circle one): Yes No

If **FEMALE**; Are you currently pregnant? (Circle one): Yes No

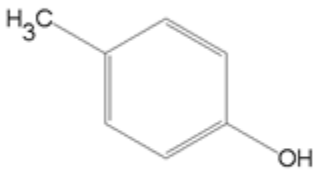
If **FEMALE**; Are you in menopause or post menopause? (Circle one): Yes No

APPENDIX D

VANILLIN AND P-CRESOL



VANILLIN



P-CRESOL

APPENDIX E

BLOOD ANALYSIS

Blood samples were collected by Blood Assurance and refrigerated at 4 degrees Celsius immediately after collection until filtration. One milliliter aliquots of each sample were withdrawn using a sterilized syringe and needle and subjected to ultracentrifugation in a Centrifree Micropartition device with a 30,000 Dalton cut-off membrane at 2000x*g* for 30 minutes (De Smet *et al.*, 1998). Filtrate samples containing small molecules were labeled and kept refrigerated prior to further analysis.

Standard solutions containing single analyte molecules to be detected that were dissolved in HPLC-grade water at varying concentrations were examined in order to verify the detection (retention times, corresponding peak height, and mass spectra) of these molecules using the HPLC and MS techniques described below. These included analysis of p-cresol (4 μM, 104 μM, 204 μM, 304 μM, 404 μM, 504 μM, 604 μM, 804 μM, 904 μM, 1104 μM, 1204 μM, 1304 μM, 1404 μM, 1504 μM, 1604 μM, 1704 μM, and 2300 μM), vanillin (150 μM, 1500 μM, and 3000 μM), 2-amino-p-cresol (10 μM, 200 μM, 600 μM, 1000 μM, 1400 μM, and 1800 μM), and tryptophan (150 μM, 1500 μM, and 3010 μM).

Reversed-Phase High Performance Liquid Chromatography (HPLC) with a ThermoFinnigan UV1000 SpectraSystem HPLC was used for separation of molecular components in the filtrate mixture. The instrument was equipped with a stationary phase 250-mm x 4.6-mm C18 column (void volume of 2.5 mL); mobile-phase buffers used in the separation were 50mM ammonium formate (HCO_2NH_4) made in HPLC-grade water (buffer A) and HPLC-grade methanol (CH_3OH, buffer B) (as described in De Smet *et al.* 1998). A five minute column pre-clean was performed using methanol prior to each set of analyses, and the sample syringe was washed three times with methanol and water between injections to avoid cross-contamination.

Filtrate samples (20μL) were loaded onto the column and subjected to elution over 18 minutes at a flow rate of 1 mL/min by the mobile phase buffers as follows: gradient elution was initialized at 100% HCO_2NH_4 / 0% CH_3OH. Over the first three minutes of each run, buffer concentrations shifted from 0-40% CH_3OH; for the next ten minutes, the gradient changed from 40-75% CH_3OH; the final two minutes of each run ramped the concentration of CH_3OH to 100%. Components of filtrate samples eluting from the column were detected by ultraviolet absorbance using a deuterium lamp at a wavelength of 280 nm. Absorbance was also monitored at a wavelength of 214 nm, but no significant differences were observed in the elution chromatograms compared to the profile with detection at 280 nm. Eluent fractions corresponding to each portion of the HPLC chromatogram were collected in labeled test tubes.

Mass spectrometry (MS) experiments were performed for each sample fraction collected from the HPLC to determine the content of the molecules contained in each, thus allowing identification of the presence of uremic toxins. Data were collected using a ThermoFinnigan

Polaris Q MS equipped with a Direct Probe Controller. All samples were mixed thoroughly and the syringe cleaned three times with methanol before deposition of 6-8 μL of sample onto the probe filament. Samples were and allowed to air-dry, then heated in vacuum to remove remaining solvent. Each experiment consisted of a temperature ramp for ballistic heating of the filament from 0-1000 mA/sec at 200 mA/sec in order to desorb the sample into the MS source. Samples were then held at 1000mA/s for 120 seconds, and ions were detected in the molecular weight range of 0-300 amu in order to identify uremic toxin molecules. Immediate cleaning of the filament was performed by the instrument after each run.

APPENDIX F

IRB APPROVAL LETTER

MEMORANDUM

TO: Jessica McKinney **IRB # 12- 149**

Dr. Nicky Ozbek

FROM: Lindsay Pardue, Director of Research Integrity

Dr. Bart Weathington, IRB Committee Chair

DATE: October 18, 2012

SUBJECT: IRB # 12-149: Odor Sensitivity and the presence of P-cresol

The Institutional Review Board has reviewed and approved your application and assigned you the IRB number listed above. You must include the following approval statement on research materials seen by participants and used in research reports:

The Institutional Review Board of the University of Tennessee at Chattanooga (FWA00004149) has approved this research project #12-149.

Please remember that you must complete a Certification for Changes, Annual Review, or Project Termination/Completion Form when the project is completed or provide an annual report if the project takes over one year to complete. The IRB Committee will make every effort to remind you prior to your anniversary date; however, it is your responsibility to ensure that this additional step is satisfied.

Please remember to contact the IRB Committee immediately and submit a new project proposal for review if significant changes occur in your research design or in any instruments used in conducting the study. You should also contact the IRB Committee immediately if you encounter any adverse effects during your project that pose a risk to your subjects.

For any additional information, please consult our web page http://www.utc.edu/irb or email instrb@utc.edu

Best wishes for a successful research project.

VITA

Jessica McKinney is a Tennessee native who completed her Bachelor of Science degree at the University of Tennessee-Chattanooga in Psychology with a minor in Biology. She originally moved to Tennessee through her father's career in the military, which inspired her research interest in PTSD. While at the University of Tennessee at Chattanooga, she has presented at multiple conferences and worked with different research teams. She will be continuing her education by pursuing a PhD in Clinical Psychology in hopes to work with the military.